Aggrecanase did not appear to play a major role in regulating aging-related degeneration in the soft tissues of the IVD, possibly the result of a slower remodeling or lower aggrecan content for these regions. Future studies of older mouse spines would be useful to determine if these changes progress or stabilize with aging-related degeneration.

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TRANSCRIPTIONAL INDUCTION OF TYPE X COLLAGEN EXPRESSION AND HYPERTROPHIC DIFFERENTIATION OF CHONDROCYTES BY RUNX2 DURING OSTEOARTHRITIS PROGRESSION

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Purpose: Hypertrophic differentiation of chondrocytes is essential not only for physiological skeletal development and growth, but also for pathological disorders like osteoarthritis (OA). This study investigated the molecular mechanism underlying transcriptional regulation of type X collagen (COL10), the representative marker of hypertrophic differentiation, during OA progression.
Methods: We created an experimental mouse OA model by producing instability in the knee joint through surgical resection of the medial collateral ligament and meniscus. Expressions of matrix proteins, proteinases, and transcription factors were then examined by immunostainings and real-time RT-PCR in the medial cartilage of knee joints during the progression of cartilage degradation. To know the physiological role of a transcription factor Runx2, we compared the OA progression in heterozygous Runx2-deficient mice and the wild-type littermates. We further established stable lines of mouse chondrogenic ATDC5 cells overexpressing Runx2 and dominant negative Runx2 through retroviral transfection, and examined the differentiation by real-time RT-PCR for COL10 mRNA level, and by Alizarin-red and von Kossa stainings. Promoter activity of COL10 was determined by luciferase assays using HeLa cells transfected with a luciferase-reporter gene construct containing a 4.5-kb fragment of the human COL10 promoter. To confirm the specific binding of the identified core responsive region, we performed electrophoretic mobility shift assay using nuclear extracts from Runx2-overexpressed COS7 cells.
Results: Runx2 expression was induced in chondrocytes at the superficial and middle layers of joint cartilage as early as 2 weeks after the knee joint instability, which was not observed in the sham-operated joint. Most of the Runx2-positive chondrocytes then expressed COL10 at 2-4 weeks, and thereafter produced collagenases causing degradation of the cartilage matrix. In heterozygous Runx2-deficient mice, however, COL10 expression, as well as the subsequent collagenase expression and matrix degradation, was suppressed under the OA induction, indicating that Runx2 contributes to the pathogenesis of OA through chondrocyte hypertrophy. In the ATDC5 cell culture, COL10 expression, Alizarin-red and von Kossa stainings were markedly increased by the Runx2 overexpression, whereas they were decreased by the dominant negative Runx2 overexpression. The COL10 promoter activity was enhanced by Runx2 transfection, and deletion analysis using a series of 5'-deletion constructs of the promoter identified the core responsive element to Runx2 around the -80 bp region (HY-box). The transcriptional activity of Runx2 was suppressed by a site-directed mutagenesis in the HY-box. The tandem-repeat constructs responded to Runx2 depending on its repeat number. The wild-type HY-box oligonucleotide probe, but not with the mutated probe, showed specific binding with nuclear extracts from Runx2-overexpressed COS7 cells. Cold competition with an excess of unlabelled wild-type probe, but not with the unlabelled mutated probe, suppressed formation of the complex. In addition, the complex underwent a supershift by the antibody to Runx2, confirming the specific binding of the HY-box and Runx2.
Conclusions: We demonstrated the contribution of Runx2 to chondrocyte hypertrophy with COL10 induction during OA progression, and identified the core responsive region HY-box in the human COL10 promoter. Studies on molecules related to Runx2/HY-box will lead to elucidation of the molecular network underlying chondrocyte hypertrophy and OA pathogenesis.

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EXTREME OBESITY DUE TO IMPAIRED LEPTIN SIGNALING DOES NOT CAUSE OSTEOARTHRITIS IN MICE

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Purpose: Osteoarthritis (OA) is strongly associated with obesity. Both leptin deficient (*ob/ob*) and leptin receptor deficient (*db/db*) mice develop morbid obesity, providing models in which to explore the relationship between obesity and OA. However, the pro-inflammatory effects of leptin and its association with OA suggest that impairing leptin signaling may mitigate against joint degeneration. We studied *ob/ob*, *db/db*, and wild-type (*WT*) mice to determine whether leptin-impaired mice develop OA using histological and microCT analysis. We also examined the effect of leptin signaling on serum cytokine levels.
Methods: All analyses were performed on 44 wk-old female *WT* (C57BL/6J; n=4), *ob/ob* (B6.V-Lep^{ob}/J; n=6), and *db/db* (B6.Cg-m +/+ Lepr^{db}/J; n=5) mice (Jackson Laboratories). Percent body fat was determined by DEXA, and subchondral bone thickness, and trabecular bone fraction and density of the tibial epiphysis of the knees were determined by microCT. Sagittal histological sections of knee joints were then scored using a modified Mankin scoring system as follows: articular cartilage structure (0-11), Safranin-O staining (0-8), tidemark duplication (0-3), fibrocartilage (0-2), chondrocyte clones in uncalcified cartilage (0-2), hypertrophic chondrocytes (0-2), and relative subchondral bone thickness (0-2) for a maximum score of 30 per location. 5 blinded graders scored sections separately for the medial and lateral femoral condyles and tibial plateau. Levels of serum leptin and hyaluronic acid (HA) were quantified by ELISA. The following serum cytokines and chemokines were measured using a custom multiplex bead immunoassay (Biosource), specific to mouse, with the Luminex 100 instrument: GM-CSF, IL-1α, IL-1β, IL-6, IL-17, KC, RANTES, and TNF-α. A nested 2-level analysis of variance (ANOVA) was used to determine statistical significance due to either impaired leptin signaling (pooled *ob/ob* and *db/db* vs. *WT*) or strain (*ob/ob* vs. *db/db*).
Results: *ob/ob* and *db/db* mice had $>$3-fold increase in body mass and $>$8-fold increase in body fat compared to *WT* mice ($p<0.01$). Despite this extreme adiposity, mice with impaired leptin signaling did not exhibit increased articular cartilage degeneration. Mankin OA scores were not significantly different for the total joint ($p=0.67$) or for any of the joint locations ($p>0.27$). *ob/ob* and *db/db* mice had significantly reduced tibial (Fig. 1), but not femoral ($p=0.20$), subchondral bone thickness. The reduced thickness was offset by an increased relative trabecular bone fraction of the tibial epiphysis (Fig. 1), resulting in a similar total epiphyseal bone volume (Fig. 1). Leptin levels were consistent with expected phenotypes for the mice (Table 1). Cytokine, chemokine, and HA levels were not significantly affected by impaired leptin signaling, except for KC (analog to IL-8 in humans), which was elevated in *ob/ob* and *db/db* mice (Table 1).
Conclusions: Despite becoming extremely obese with slightly